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- Published:**
- with international search report
  - before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- (88) Date of publication of the international search report: 31 December 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

A3

WO 2003/087774

(54) Title: ECIST MICROARRAYS FOR DUAL SCREENING OF DNA HYPERMETHYLATION AND GENE SILENCING

(57) Abstract: Novel methods are herein provided for high-throughput, dual analysis of DNA methylation and gene expression, and triple analysis of DNA methylation, gene expression and gene-associated histone acetylation in cancer cells using arrayed expressed CpG island sequence tags (ECISTS). ECISTS correspond to genomic DNA fragments comprising GC-rich segments along with promoter and/or exon (e.g., first exon) portions of genes. The GC-rich segments are useful for screening hypermethylated CpG sites in cancer cells, while the corresponding promoter and exon-containing portions are useful for determining corresponding transcript levels and assessing histone acetylation. Also provided are high-throughput methods for either confirming methylation-dependent gene silencing, or identifying therapeutically effective demethylating agents, using the ECIST array panels to identify hypermethylated loci, and measure expression levels thereof after cellular exposure to demethylating agents. Further provided are high-throughput methods for distinguishing between direct (primary) demethylation-dependent gene up-regulation, and indirect (secondary) demethylation-dependent up-regulation within apparent epigenetic cascades.

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US03/11598

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : C12Q 1/00, 1/68; C12P 19/34; C07H 21/02 21/04  
US CL : 435/4, 6, 91.1; 536/23.1, 23.5, 24.1, 24.3, 24.33

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
U.S. : 435/4, 6, 91.1; 536/23.1, 23.5, 24.1, 24.3, 24.33

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Continuation Sheet

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HUANG et al. Methylation Profiling of CpG Islands in Human Breast Cancer Cells. Hum. Mol. Gen. 1999, Vol. 8, No. 3, pages 459-470, see whole document.	1-52
Y	YAN et al. CpG Island Arrays: An Application Toward Deciphering Epigenetic Signatures of Breast Cancer. Clin. Canc. Res. April 2000, Vol. 6, pages 1432-1438, see whole document.	1-52
Y	AHL UWALIA et al. DNA Methylation and Ovarian Cancer: I. Analysis of CpG Island Hypermethylation in Human Ovarian Cancer Using Differential Methylation Hybridization. Gynecologic Oncology. 2001, Vol. 82, No. 2, pages 261-268, see whole document.	1-52
Y	YAN et al. Dissecting Complex Epigenetic Alterations in Breast Cancer Using CpG Island Microarrays. Cancer Res. 01 December 2001, Vol. 61, pages 8375-8380, see whole document.	1-52

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"B" earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	"A" document member of the same patent family

Date of the actual completion of the international search

27 July 2003 (27.07.2003)

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INTERNATIONAL SEARCH REPORT

PCT/US03/11598

**Continuation of Item 4 of the first sheet:**

The title is too long, PCT Rule 4.3, suggested new title follows: "ECIST MICROARRAYS FOR DUAL SCREENING OF DNA HYPERMETHYLATION AND GENE SILENCING".

**Continuation of B. FIELDS SEARCHED Item 3:**

Databases: USPAT, PGPUB, JPO, EPO, Derwent, Medline, Biosis, Embase, CaPlus, Biotechds

Search Terms: CpG, probe, array, microarray, chip, methylation, label, tissue, gene, CpG island

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